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14. ABSTRACT

Semaphorin 3B is a secreted member of the semaphorin family, important in axonal guidance, which we and others have recently shown can act as a tumor suppressor by inducing apoptosis either by re-expression in tumor cells or applied as a soluble ligand. The common method of inactivation of SEMA3B is by allele loss and HDAC. We study the mechanism of SEMA3B effect on breast cancer cells. We found that vitamin D3 and TGFβ1 can induce SEMA3B protein and promoter activation. We found that VEGF165, which is reported to bind to neuropilin (NP), significantly decreased the anti-mitotic effect of transfected or secreted SEMA3B on breast cancer cells. By contrast, VEGF121, a VEGF variant that lacks binding to NPs receptors, had no effect on SEMA3B growth suppressing activities. In addition, SEMA3B competed for binding of 125I-VEGF165 to lung and breast cancer cells. We also found that SEMA3B apoptotic and anti-proliferative effect on cancer lines it is in part by the inhibition of Akt pathway. In conclusion, we hypothesize that VEGF165, produced by tumor cells, acts as an autocrine survival factor and SEMA3B mediates its tumor suppressing effects, at least in part, by blocking this VEGF autocrine activity and by inhibiting the Akt pathway.

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tumor suppressor gene, breast cancer and apoptosis

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Introduction

Semaphorin 3B (*SEMA3B*) is located at 3p21.3, a site of very frequent allele loss in the early pathogenesis of lung and breast cancer (1-3). *SEMA3B*, encodes a secreted protein with tumor suppressor activity for lung cancer (4). Treatment with exogenously added SEMA3B or introduction of a plasmid encoding *SEMA3B* into H1299 non-small cell lung cancer (NSCLC) cells lead to induction of apoptosis and a dramatic decrease in colony formation (3, 4). Independent studies in ovarian cancer by Tse et al. (5) demonstrated that SEMA3B also inhibited ovarian tumor formation in a xenograft model. We previously hypothesize that inactivation of SEMA3B is a common and early even in breast and other cancers. Even further we hypothesize that SEMA3B exerts its tumor suppressor function through competing with breast cancer produced VEGF (vascular endothelial growth factor) acting as a tumor survival factor. If SEMA3B functions as a tumor suppressor that undergoes inactivation by mutation, LOH, promoter methylation or lost of receptors, it could provide both a diagnostic tool and as a soluble molecule a new treatment breast and other cancers with similar lost of SEMA3B.

Body

Research accomplishments associated with each task.

Task 1. Verification of tumor suppressor activity of SEMA3B in breast cancer cells.

Preparations of different expression vectors, pSEMA3B, Flag-SEMA3B, SEMA3B tumor acquired missense mutant and p53 have been accomplished. Proliferation studies in breast cancer cell lines are in process. We have observed 50-90% reduction in cell proliferation in around 75% breast cancer cell lines treated with Cos7 media after transfection with SEMA3B, or control vector (Figure 1). It is important to point out that the lung cancer line H1299 is used as a positive control for SEMA3B effect. The lung line H2009 which express endogenous levels of SEMA3B it was used as a negative control for SEMA3B effect. In conclusion we have found that most cells lines will respond to SEMA3B growth inhibition.

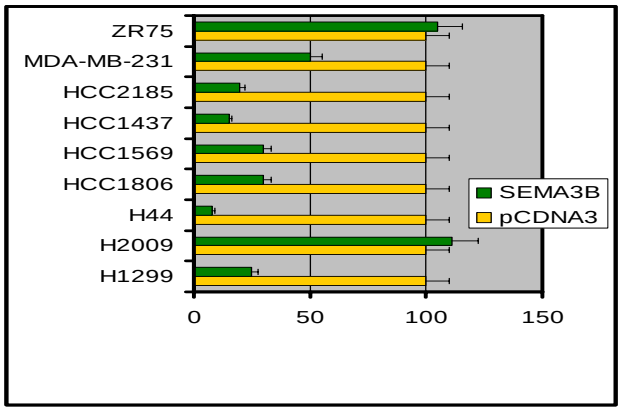

Figure 1. Example of effect of SEMA3B in cell proliferation in breast cancer cells lines. Cells were counted after 5 days of treated with SEMA3B or control media.

Task 2. Study the expression and promoter methylation patterns of SEMA3B using a panel of breast cancer cell lines.

We used western blot analysis to examine the expression of SEMA3B in a panel of breast cancer lines; representative examples are shown in Figure 2. We have developed a SEMA3B antibody that can detect transfected protein and it is used as a blocking antibody against SEMA3B. We have also developed a

monoclonal antibody that can detect endogenous levels of the SEMA3B protein. We used H2009 cells as a positive control for SEMA3B expression. We verified that the monoclonal antibody was targeting SEMA3B by using siRNA. After 72 hour post transfection of siRNA targeting SEMA3B we observed a more than 80% decreased in SEMA3B protein band intensity in H2009. In a panel of breast cancer lines for the presence of endogenous SEMA3B we found that only 20% express the endogenous protein (Figure 2). These data correlated with lung cancer cell lines data in which SEMA3B is expressed in less than 15% of the cell lines.

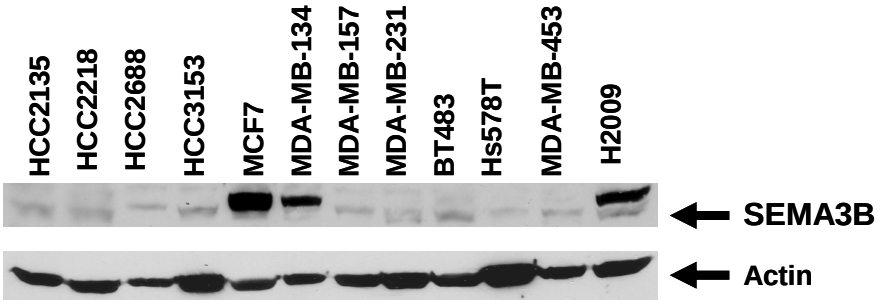

Figure 2. Example of expression of endogenous SEMA3B in a panel of breast cancer cells by western blot analysis. We used the mouse monoclonal antibody with epitope that binds to CALQSLPLESRRKGRNRRTHAPEP.

While some previous studies have shown the SEMA3B has some methylated CpG sites in its promoter, it does not have a well defined CpG island near the 5' end of the locus. We analyzed a panel of breast cancer, lung cancer and normal lung (HBEC) cell lines before and after 5-aza treatment (1 μM and 100 μM) and found that all the cell lines with the exception of HCC3153 express detectable levels of SEMA3B mRNA (Figure 3). It is possible that SEMA3B exhibits differential methylation of certain CpG sites near its transcription start site and that this may vary with passage in culture and culture conditions. The absence of a 5' CpG island leads to the conclusion that inhibition of SEMA3B expression occurs through mechanism independent of DNA methylation. We decide to look at histone acetylation. Acetylation of the lysine residues at the N terminus of histone proteins removes positive charges, thereby reducing the affinity between histones and DNA. This makes RNA polymerase and transcription factors easier to access the promoter region. Therefore, in most cases, histone acetylation enhances transcription while histone deacetylation represses transcription.

Histone deactylate inhibitors mediate growth inhibition and apoptosis in cancer cells. They have been shown to regulate the transcription of a defined set of genes through chromatin remodeling and might also modulate the acetylation status of a series of non-histone targets. HDAC inhibitors have entered clinical trials for solid and liquid tumors. We pursue studies in the effect of the HDAC, TSA in SEMA3B protein level. Cells were treated with TSA and collected every 24 hour for 72 hours. We observed that TSA could induce SEMA3B protein levels in the breast cancer line MCF7 after 72 hours of treatment (Figure 4). Similar effect was observed in other breast and lung cancer cell lines. Furthermore, we observed that HDAC activity was unchanged by SEMA3B treatment in MDA-MB-231

and MCF-7 breast cancer cells (data not shown). Taken together we conclude that SEMA3B gene expression is control by other mechanism independent of DNA methylation, but thru histone acetylation.

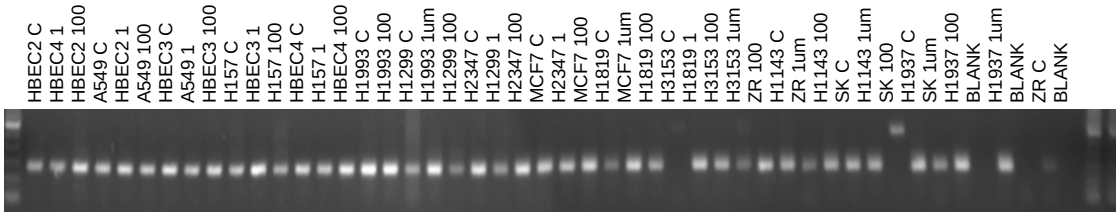

Figure 3. SEMA3B RT-PCR in breast and lung cancer cell lines after 5-aza treatment. Cells were treated with 5-aza at concentration of 1 μM and 100 μM. mRNA was collected after treatment and RT-PCR was run for SEMA3B and GAPDH (data not shown). 5-aza was used to remove the methylation in the DNA and will aloud re expression of the gene being methylated when silencing is due to methylation.

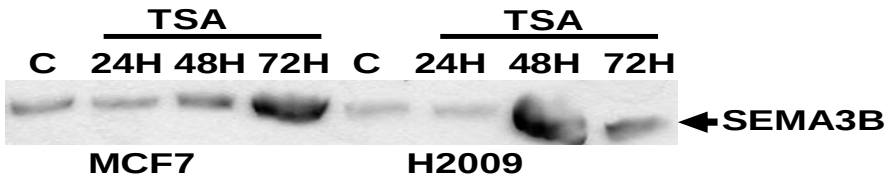

Figure 4. Western blot analysis for SEMA3B expression after treatment with TSA for MCF-7 breast cancer and H2009 breast cancer cells. Cells were seeded and treated with TSA and pellets were collected every 24 hours. We observe induction by the histone deacetylated inhibitor, TSA at 72H for MCF-7 and 48H for H2009. TSA = trichostatin A

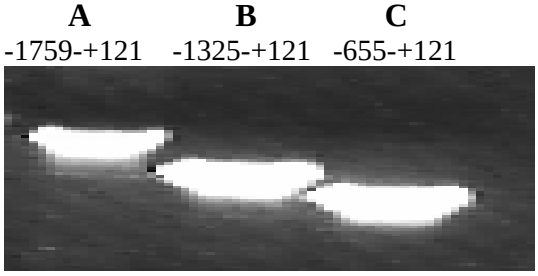

Figure 5. PCR for SEMA3B promoter inserts using the cosmic plasmid LUCA14 that contains part of the 3p21 region which encode SEMA3B.

Task3. Study the SEMA3B promoter region and mechanism of its transcriptional activation.

We have made three SEMA3B promoter constructs in a pGL3 luciferase reporter vector. For the synthesis of SEMA3B promoter we used the cosmic plasmid LUCA14 (Figure 5) SEMA3B is a very C-rich promoter making its synthesis challenging.

Vitamin D3 and SEMA3B expression: Vitamin D3 or 1α,25-dihydroxyvitamin D3 (VD3) compounds have been demonstrated to be effective for inhibition of cell proliferation and induction of apoptosis in vitro and in vivo in a variety of cancer cell models. They are currently in clinical trials for cancer therapeutics**.** Althuogh the apoptosis induced by VD3 compounds is characterized by genomic DNA fragmentation, caspase activation and poly(ADP-ribose) polymerase cleavage, the mechanism by which these events happen is not very clear. For example, it was shown that Bcl-2 expression could be positively or negatively regulated by VD3 compounds, but not essential for apoptosis. These results suggest that other factors or targets rather than Bcl-2 may be involved in the VD3 compound-induced growth inhibition and apoptosis. Recently it was found that in squamous carcinoma cells showed that the active form of Vitamin D3, VD3 and its receptor (VDR) is involve in SEAM3B transcriptional activation. MCF-7 treated with VD3 showed an increased in SEMA3B protein after 48H (Figure 6), however in other experiments we has observed induction of SEMA3B protein as early as 6 hours (9). In western blot analysis we found that VDR is expressed in 65% of breast cancer lines (data not shown). MCF-7 showed endogenous expression of VDR and in proliferation studies we found a significant decreased in proliferation after treatment with VD3 starting at concentration as low as 1 nM (Figure 7). In promoter studies for SEMA3B gene we used three different constructs -1759-+121, -1325-+121, -655-+121 links to a luciferase reported gene. We found that VD3 at 100 nM induced the three construct by 2.5 to 3.5 fold when comparing with control or untreated cells (Figure 8). In conclusion we could verified that VD3 can induced SEMA3B protein levels in the breast cancer line MCF7 and the lung cancer line H2009 both lines are know to express endogenous SEMA3B.

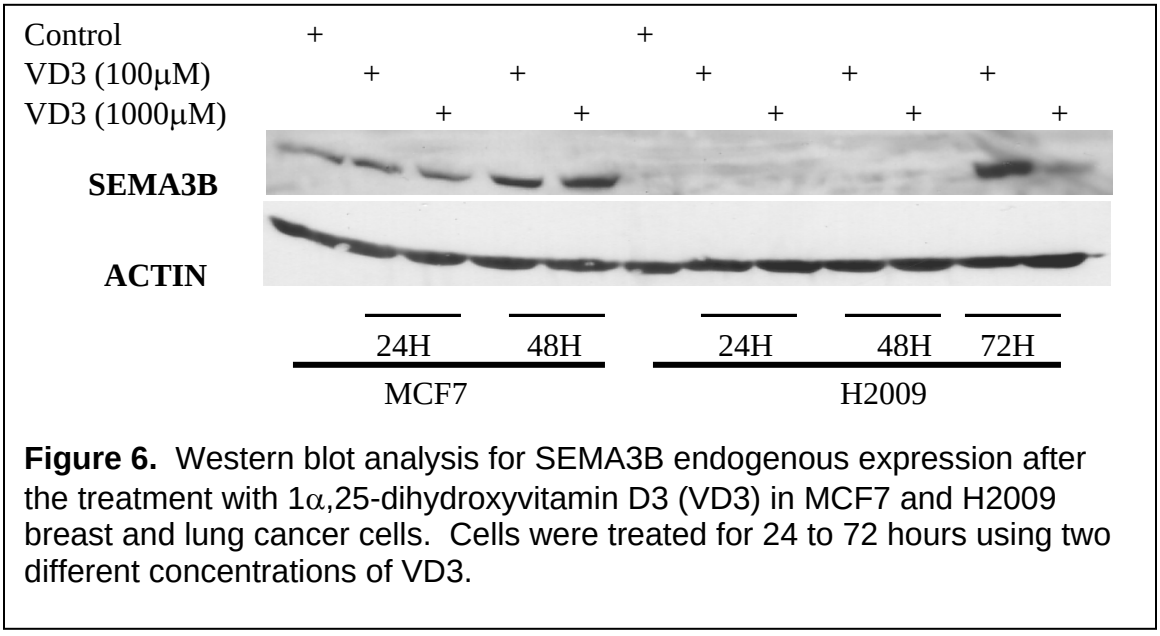

Figure 6. Western blot analysis for SEMA3B endogenous expression after the treatment with 1α,25-dihydroxyvitamin D3 (VD3) in MCF7 and H2009 breast and lung cancer cells. Cells were treated for 24 to 72 hours using two different concentrations of VD3.

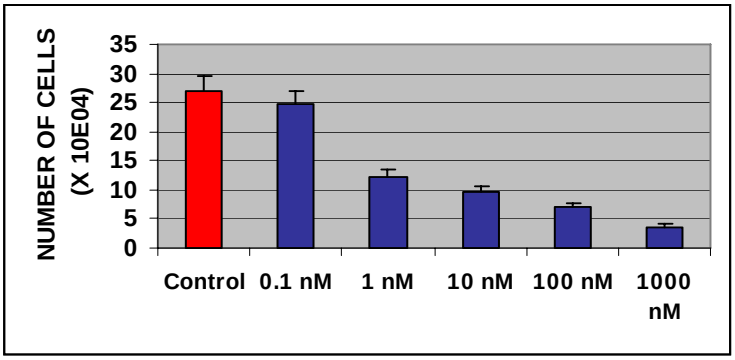

Figure 7. Cell proliferation assay for MCF-7. Cells were treated with different concentration of 1α,25-dihydroxyvitamin D3. After 6 days cells were harvest and counted.

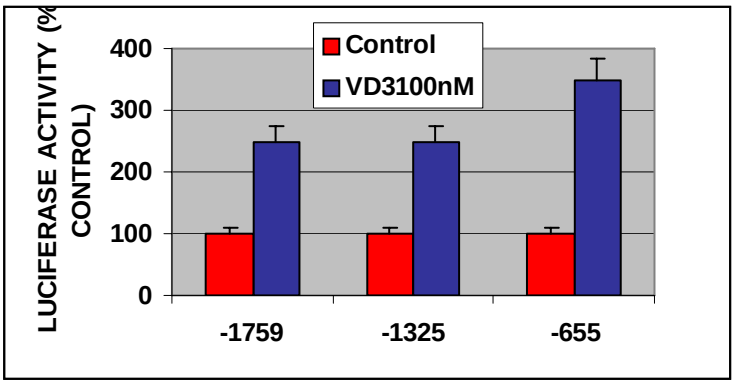

Figure 8. Deletion analysis of the SEMA3B gene promoter. Cells were transfected with the different construct containing SEMA3B gene promoter in co transfection with VDR expression vector. Cells were treated for 48H with 1α,25-dihydroxyvitamin D3 (100 nM). Cells were harvest and luciferase activity was detected by using a luminometer.

TGFβ1 and SEMA3B expression: TGFβ1 is a molecule that is fundamental for the maintenance of the homeostasis between cell growth and apoptosis. The loss of the ability to respond to the growth inhibitory effect of TGFβ1 may convey a growth advantage to the malignant cells over the surrounding normal cells; changes in responsiveness to TGFβ1 have been linked to malignant transformation, tumor progression and tumor regression. We found that TGFβ1 can induced or reduce levels of SEMA3B protein. In MCF-7 we found that TGFβ1 reduced SEMA3B protein levels, but in H1299 a cell sensitive to SEMA3B treatment we observed a up regulation in SEMA3B protein (Figure 9). The increase in protein also was translated to promoter induction of SEMA3B or in other words transcriptional activity. We also found that SEMA3B treatment in sensitive cells translate to inhibition of Smad-2 phosphorylation giving us the indication of a possible loop between TGFβ1 and SEMA3B.

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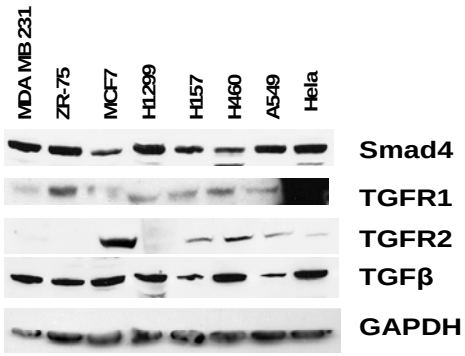

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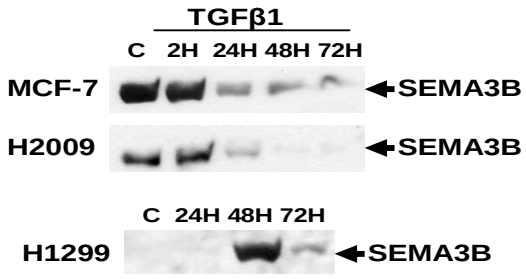

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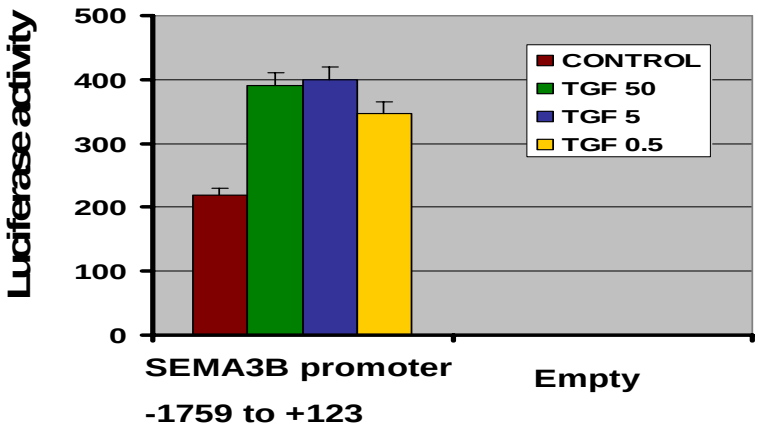

Figure 9. TGF-beta 1 effect on SEMA3B protein levels. A. A panel of different types of cells from breast cancer, lung and cervical cancer where screen fro the different TGFRs and TGF-beta. B. Cells were treated with TGF-beta1 and induction of SEMA3B was observed in cells sensitive to SEMA3B effect, but not in cell resistant such as H2009 and MCF7. C. Promoter studies for SEMA3B after treatment with TGFβ1. Induction in promoter activation correlated with protein expression and SEMA3B sensitivity in different cell lines.

Taken together the data connect the gene regulation of SEMA3B by the two controversial apoptotic pathways VD3-VDR and TGFβ1 that are being consider for therapeutic development.

Task 4. Study of the pathways and mechanism of tumor suppressor activity of SEMA3B in breast cancer cells.

Extensive work has gone to this task. Previous research has shown that heparin binding domain containing VEGF-A isoforms (145, 165, 189, but not 121) can bind to neuropilins (9). Neuropilins are the receptors for class 3 semaphorin (SEMA3B is part of this class). Further more resent research has shown that SEMA3A and $VEGF_{165}$ compete for binding with neuropilins. Firstly we determined the role of VEGF-A in the survival of H1299 lung and MDA-MB-231 breast cancer cell lines. We have used a anti-VEGF-A neutralizing antibody. Using these techniques that neutralize the biological activity of VEGF-A, we observed a 40-80% decrease in cancer cell proliferation after four-five days of treatment (Figure. 10). These data suggest that tumor cell produced VEGF has an important role in the survival and or growth of H1299 lung and MDA-MB-231 breast cancer lines.

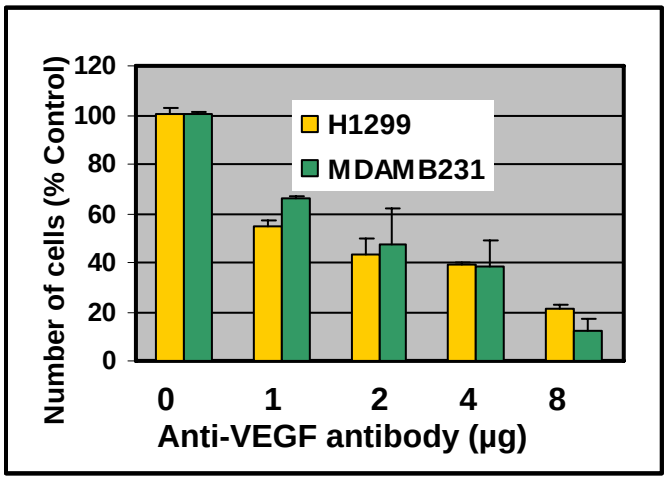

Figure 10. VEGF-A play a role in the survival of H1299 lung and MDA-MB-231 breast cancer cells. H1299 and MDA-MB-231 were seeded and treated with different doses of anti-VEGF monoclonal antibody. Cells were trypsinize and count after 4-5 days. The cell proliferation inhibition by the antibody was dose dependent.

Secondly we determined that SEMA3B inhibits $^{125}I\text{-}VEGF_{165}$ binding to H1299 and MDA-MB-231 cancer cells. It is important to mention that our source of SEMA3B is from Cos7 cells medium after transfection with SEMA3B expression vector. Our control is the medium after Cos7 transfection with empty vector. Cell surface binding of $^{125}I\text{-}VEGF_{165}$ was tested on both cell lines with and without the addition of SEMA3B. Specific $^{125}I\text{-}VEGF_{165}$ binding was observed in both cell lines after two hour of incubation with 100 pM of labeled $VEGF_{165}$ (Fig. 11) SEMA3B, decreased the specific binding of $^{125}I\text{-}VEGF_{165}$ of all cell lines (Fig.11) with maximal inhibition of 50% for MDA-MB-231 and 65% for H1299, respectively. By contrast, Cos7 control had no significant effect on $^{125}I\text{-}VEGF_{165}$ binding (Fig. 11). These data suggest that $VEGF_{165}$ and SEMA3B share binding sites on these human tumor cells as was previously observed for SEMA3A and $VEGF_{165}$ on NP receptor.

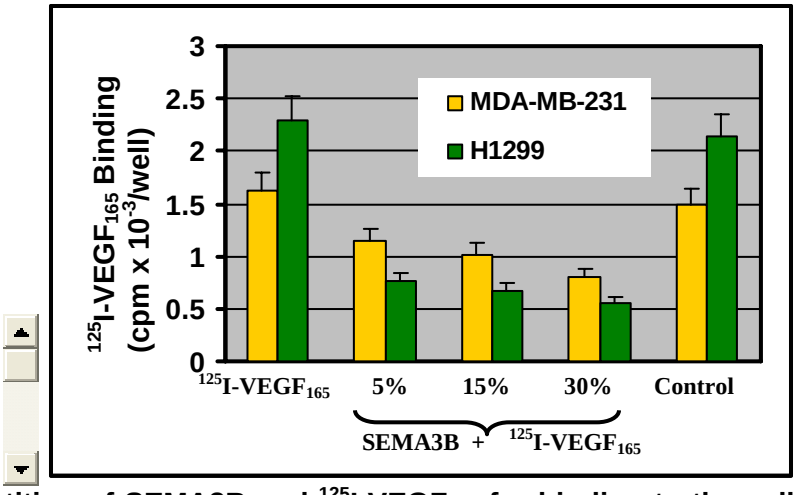

Figure 11. Competition of SEMA3B and ^{125}I-VEGF$_{165}$ for binding to the cell surface receptors on MDA-MB-231 and H1299 cells. MDA-MB-231 and H1299 were incubated for 2 hours with ^{125}I-VEGF$_{165}$ and SEMA3B in the binding buffer and the bound ^{125}I-VEGF$_{165}$ was detected using gamma counter. ^{125}I-VEGF$_{165}$ binding with SEMA3B added at 5, 15 and 30% of total volumen per well (200 µl) (lanes 2-4), which conditioned medium from the Cos-7 cells transfected with empty vector served as a control. Non-specific binding was determined in the presence of 100 fold molar excess of unlabeled VEGF unlabeled and was subtracted from the experimental values.

Thirdly, we determined that VEGF$_{165}$ antagonizes SEMA3B mediated effects on tumor cell proliferation. SEMA3B inhibited proliferation of H1299 and MDA-MB-231 cells by 50% compared with vector control while rabbit anti-SEMA3B antibodies neutralized this effect (Table 1). Control IgG from pre-immune rabbits had no effect on the inhibitory effect of the SEMA3B or vector control (data not shown). These results demonstrate that SEMA3B protein present in the control of SEMA3B transfected Cos7 cells, is responsible for the anti-proliferative effect. Treatment with mutant SEMA3B (SEMA3Bmut1) vector showed no effect in cell number when compared with control. The anti-proliferative effect of SEMA3B was overridden by co-treatment with of VEGF$_{165}$. By contrast, co-treatment with VEGF$_{121}$, which does not bind to NP-1 and NP-2, had no significant effect on cell proliferation as compared to SEMA3B alone. Transfection with VEGF$_{121}$ or VEGF$_{165}$ alone did not affect cell proliferation in the absence of SEMA3B. The specificity of the VEGF$_{165}$ reversal of SEMA3B growth inhibition was confirmed by treating with monoclonal anti-VEGF antibody 2C3 which detects VEGF bound to its receptors (10) which gave similar cell numbers as treatment with SEMA3B alone. In contract to the anti-VEGF neutralizing antibody the anti-VEGF monoclonal antibody 2C3 does not neutralize endogenous VEGF-A and no inhibition of growth was seen. The lacks of effect of VEGF$_{121}$ indicates that only a neuropilin-binding VEGF isoform (VEGF$_{165}$) is able to counteract the effects of SEMA3B. Taken together, these data suggest that SEMA3B is a potent growth inhibitory factor for human lung cancer H1299 and breast cancer MDA-MB-231 cells, and that VEGF$_{165}$ but not the VEGF$_{121}$ isoform, overcomes the SEMA3B growth inhibitory effect.

Although the pro-apoptotic and growth inhibiting activities of SEMA3B in epithelial cancer cells have been demonstrated in several laboratories, the signal transduction pathway used for these actions have not yet been established. Recent studies demonstrating counter-balancing relationships between VEGF and SEMA3B or SEMA3B and SEMA3F (5,11), suggest involvement of the PI3K/Akt pathway because it is a major pathway activated by VEGF. We tested two breast cancer lines, MDA-MB-231 and ZR75. MDA-MB231 is a SEMA3B sensitive while ZR75 is SEMA3B resistant in proliferation studies

explained at least in part by its failure to express neuropilins (Fig. 1). Treatment with SEMA3B significantly reduced phosphorylation of Akt at Ser 473 as shown in MDA-MB-231 but not in the NP negative ZR-75 (Fig. 12). The reduction in phospho-Akt at Ser 473 was observed from 1 to 24 hours after SEMA3B-CM exposure in MDA-MB-231 cells. We also assessed the effect on phosphorylation of SEMA3B in two proteins involved in the PI3K/Akt pathway PDK1 and GSK-3β. We observed similar patterns of phosphorylation as those observed in Akt for MDA-MB-231 (Figure 12).. Together, these studies indicate that treatment with soluble SEMA3B inhibits the phosphorylation status and the activity of Akt in SEMA3B sensitive cancer cell lines.

Table 1. Effect of SEMA3B-CM and VEGF isoforms 165 and 121 on proliferation in H1299 lung and MDA-MB-231 breast cancer cells.

Treatment[a]	**H1299 Cell # ($x10^{-4}$) (*P* value)**[b]	**MDA-MB-231 Cell # ($x10^{-4}$)(*P* value)**[b]
Control-CM[c]	11 ± 1.32 (-)	16 ± 1.0 (-)
SEMA3B-CM[c]	5.6 ± 0.57 (0.002)[d]	5 ± 2.0 (0.002)[d]
SEMA3Bmut1-CM[e]	11 ± 1.0 (0.0005)[f]	15 ± 0.5 (0.0005)[f]
SEMA3B-CM + anti-SEMA3B[g]	10.8 ± 1.5 (0.004)[f]	13 ± 1.0 (0.004)[f]
$VEGF_{121}$	10.7 ± 0.75 (NS)[h]	16 ± 1.0 (NS)[h]
$VEGF_{165}$	11.15 ± 0.75 (NS)[h]	16 ± 2.0 (NS)[h]
$VEGF_{121}$ + SEMA3B-CM	6 ± 1.25 (0.004)[i]	8 ± 0.3 (0.003)[i]
$VEGF_{165}$ + SEMA3B-CM	12.3 ± 1.5 (0.0002)[f]	15 ± 0.6 (0.003)[f]
$VEGF_{165}$ + anti-VEGF-A Ab	12 ± 0.9 (NS)[j]	14 ± 0.8 (NS)[j]
$VEGF_{165}$ + SEMA3B-CM + anti VEGF Ab[g]	6.6 ± 0.6 (0.001)[k]	4 ± 0.9 (0.001)[k]

[a] Conditioned medium from cells transfected with various plasmids as specified above was added to MDA-MB-231 cells. [b] P value was calculated using two-tailed student t-test. [c] Cos7 cells were transfected with vector control or with SEMA3B expression vector and conditioned medium was collected 48 hours thereafter. [d] Significant decrease as compared to Control. [e] SEMA3B-mut (D397H) is inactive. [f] significant increase as compared to SEMA3B. [g] Anti-SEMA3B or anti-VEGF-A (30 μg/ml) antibody was used as neutralizing agents for SEMA3B and $VEGF_{165}$ respectively. [h] Non significant as compared to control. [i] Significant decrease when compared to cells treated with $VEGF_{121}$. [j] No significant as compared to $VEGF_{165}$. [k] significant decrease as compared to $VEGF_{165}$ + SEMA3B.

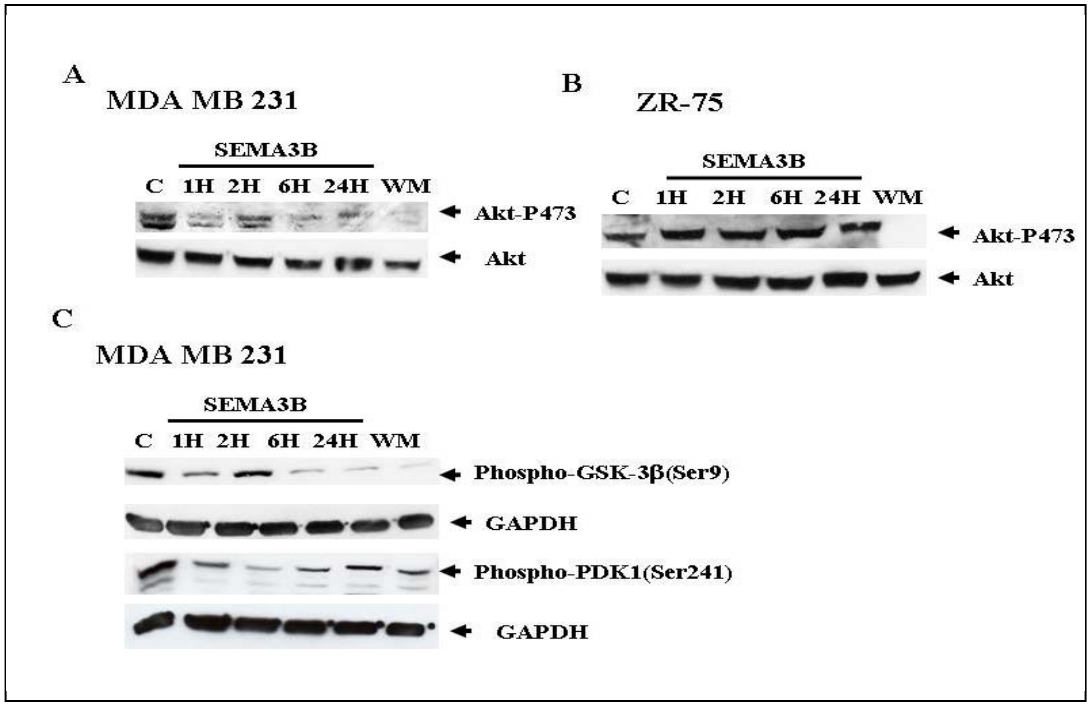

Figure 12. SEMA3B effect on Akt phosphorylation in MDA MB 231 and ZR-75 breast cancer cells**.** Cells extracts were collected after treated with Control-CM or SEMA3B-CM at different time points in MDA MB 231 and ZR-75 breast cancer cells. (A&B) Cells extracs were collected from MDA MB 231 and ZR-75 after treatment with SEMA3B-CM for 1, 2, 6 and 24 hours. Western blot analysis for phospho-Akt and whole Akt. (C) Western blot analysis using cells extracts from MDA MB 231 for PDK1 and Gsk-3β upstream and downstream protein in the Akt pathway. GAPDH was used as an internal control for loading. We used two PI3K inhibitor, wortmannin (WM) to show the Akt pathway was functional and responsive in breast cancer cells.

Key Research Accomplishments

- We made the expression plasmid for SEMA3B, SEMA3B-Flag (which it is partially inactive, SEMA3B with missense mutations (three different missense mutation found in lung cancer D397H, T415I, and D561N), and p53.
- Many breast and lung cancer lines showed a decrease in cell number after treatment with SEMA3B.
- SEMA3B mouse monoclonal antibody was made and was found to react with endogenous SEMA3B.
- SEMA3B expression is only found in 20% of breast cancer cell lines (30 lines were tested).
- SEMA3B gene silencing since to be regulated by Histone deacetylation and not by promoter methylation.

- SEMA3B gene promoter constructs were synthesis and introduce to a pGL3 luciferase reported gene vector.
- 1α,25-dihydroxyvitamin D3 induced SEMA3B in MCF7 breast cancer cells
- Vitamin D receptor is expressed in 65% of breast cancer cells
- 1α,25-dihydroxyvitamin D3 decreased cell number in proliferation studies in MCF-7 breast cancer cells
- 1α,25-dihydroxyvitamin D3 induces SEMA3B promoter up to 4 folds
- TGFβ1 induces SEMA3B protein expression
- TGFβ1 showed SEMA3B promoter induction by two folds MCF-7 breast cancer cells
- SEMA3B protein levels are induced after UVB exposure in MCF-7 breast cancer cells
- Neutralization of VEGF-A using anti-VEGF antibody produced a decrease in cell number showing the importance of VEGF-A in cell survival and growth for breast and lung cancer cells.
- SEMA3B could compete for $VEGF_{165}$ cell surface binding.
- $VEGF_{165}$ could negate the anti-proliferative effect of SEMA3B in breast and lung cancer cell but not $VEGF_{121.}$
- SEMA3B treatment in the breast cancer cell line MDA-MB-231 showed a decrease in activation of the Akt pathway. However SEMA3B effects on the Akt pathway was not observed in the breast cancer cell line resistant, ZR75.

Reportable outcomes

Manuscript

Castro-Rivera E., Ran S, Minna J.D., Tumor suppressor semaphoring 3B (SEMA3B) inhibits pro-survival signal transduction of the PI3K/Akt pathway in lung and breast cancer cells. **(JBC Submission in process)**

Castro-Rivera E., and Minna J.D., Induction of SEMA3B expression by vitamin D3 in MCF-7 breast cancer cells

Publication

Castro-Rivera, E, Ran, S, Thorpe, P and Minna, JD, Semaphorin 3B (SEMA3B) induces apoptosis in lung and breast cancer while $VEGF_{165}$ antagonizes this effect. PNAS 101(31) 2004.

Conclusions

Summary

In the present work we have shown that SEMA3B is express than 20% of breast cancer cell lines and inhibit cell proliferation in most of the breast cancer tested. Vitamin D3 induces endogenous SEMA3B and this induction was translated in SEMA3B gene promoter studies in MCF-7 cells. The significant of this work is that Vitamin D3 and vitamin D3 analogs are been study for treatment of breast cancer, however vitamin D3 at higher concentration is toxic so may be SEMA3B can become an alternative for vitamin D3 and its analogs treatment. We also observed an effect by TGFβ1 in SEMA3B protein levels, however the induction or down regulation varied from cell to cell. TGFβ1 apoptotic or growth inhibitory effect has being shown to be cell specific. We also observed an induction of SEMA3B protein in after UVB radiation (data not shown) liking SEMA3B protein induction with DNA damage and free radicals.

We also have shown that neutralizing anti-VEGF antibody inhibited breast and lung cancer cell proliferation in vitro. SEMA3B inhibits $VEGF_{165}$ binding to receptors on these tumor cells and the effect can be mediated by NP receptors alone in the absence of VEGF receptor expression. These results suggest that tumor cell produced VEGF-A is a tumor cell survival or growth factor and that

SEMA3B acts through a VEGF regulated system to mediate its tumor suppressor effects. These results are significant because it is well known that tumors required angiogenesis to be able to growth and survive. Finally we have identified PI3K/Akt pathway as one of the pathways affected by SEMA3B effect on apoptosis and cell growth.

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